Testing a Microsatellite Marker for Selection of Columnar Growth Habit in Peach [*Prunus persica* (L.) Batsch]

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Abstract

Tree fruit breeding requires large numbers of seedlings in the field for selection. These trees occupy large land areas and are expensive to maintain. Most seedlings are discarded, thus the investment in these trees is lost. Selection in the greenhouse would spare much of this expense. Marker-assisted selection offers this possibility. A microsatellite marker was tested for selection of columnar (also termed "pillar" or "broomy") growth habit (*brbr*). Columnar trees are being tested for high-density production systems. Seventy-five genotypes were used for this study. These included compact (CT), dwarf (DW), standard (ST), and 2 sources of columnar; showy-flowered (SFP) with small, melting-, white-flesh fruit, and non-showy flowered (NSP) with large, non-melting-, yellow-flesh fruit. The microsatellite marker pchgms1 has been previously assigned a position 12.5 cM from the *br* locus in SFP trees. Using pchgms1 in this study, 2 fragments were obtained. One fragment was present only in SFP trees, the other was present in all other trees, including NSP. Hybrids of SFP with NSP and with all other growth habits contained both fragments. These results indicate that trees heterozygous for SFP can be selected, using pchgms1. At the seedling stage, prior to field planting, ST seedlings (*BrBr*) cannot be readily distinguished from trees heterozygous for *br* based on morphological traits. The efficiency of breeding programs developing *brbr* (columnar) or heterozygous *Brbr* (upright) trees could be improved by discarding ST trees prior to field planting. Using pchgms1, trees carrying the columnar trait, homozygous or heterozygous, could be selected from crosses using SFP as a parent. While this study demonstrates the usefulness of a satellite marker for selecting columnar growth habit in peach, the AFRS breeding program is utilizing NSP for cultivar development because of its higher fruit quality. The development of a microsatellite marker specifically for NSP or for both SFP and NSP sources of columnar growth habit is underway.

INTRODUCTION

The development of improved woody perennial tree fruits is long-term and expensive. Marker-assisted selection (MAS) offers the possibility of saving the time and expense necessary to plant and evaluate trees in the field that do not contain genes for the desired trait(s). A major goal of the peach breeding program at the USDA-ARS Appalachian Fruit Research Station is the development of columnar peach trees for high density production systems. The gene for columnar growth, variously termed "pillar" or "broomy" (*br*) expresses incomplete dominance. Trees *brbr* are columnar, *BrBr*, standard, and *Brbr* are upright (Scorza et al., 2002) (Fig. 1). After several months of growth, *BrBr* seedlings can generally be distinguished from *brbr* seedlings. It is difficult to distinguish *BrBr* trees from *Brbr* in the greenhouse, or even in the field at close within-row spacings (1-2 m) as are used in seedling plantings. In a program aimed at development of columnar (*brbr*) and upright (*Brbr*) trees it may be desirable to discard or separate-out standard trees since these will not be of desired habit (although they can serve as parents

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hybridized to columnar trees to produce upright trees). A molecular marker for *br* would allow for pre-selection of trees in the greenhouse.

MATERIALS AND METHODS

Plant Material

A total of seventy-five 5 to 8 year-old peach trees (6 cultivars and 69 different seedlings from F_1, F_2, F_3 progenies at the USDA's Appalachian Fruit Research Station, Kearneysville, West Virginia) were used in this study. Two sources of columnar growth habit were evaluated. Both were obtained from L. F. Hough [Rutgers University, New Brunswick, N.J. (deceased)]. They were originally imported from Japan. The growth form of these two columnar clones was similar. One genotype produced double showy variegated flowers (SFP) and small, bitter, melting-, white-flesh fruit. SFP appears to be similar, if not identical, to the Japanese cultivar Hoki (Yamazaki et al., 1987). The second genotype produced non-showy flowers (NSP) and yellow-, non-melting flesh fruit. The differences in fruit and flower phenotypes and the absence of information concerning their precise origins led to questions concerning the allelic identity of these two columnar genotypes. In order to test for allelism between the columnar genes in SFP and NSP, heterozygous red-leaf SFP, developed at the Appalachian Fruit Research Station, was used as a pollen parent to hybridize with NSP. Two families from NSP x SFP were evaluated. While green-leaf columnar progeny from this cross could have resulted from self-pollination of the female green-leaf NSP parent, red-leaf progeny could only result from hybridization between the red-leaf SFP and the green leaf NSP since there were no other red-leaf trees in the block or adjacent blocks. SFP, NSP, and hybrids between these phenotypes were evaluated along with other growth habits including DW (*dwdw*), CT (*Ct*__), and ST trees.

DNA Extraction

DNA was isolated from leaf tissue using method of N. Kobayashi et al. (1998). DNA was extracted from 1 g of frozen and ground tissue. The DNA extraction products were stored at 4 °C before PCR amplification.

Marker Development

Sosinski et al. (2000) reported a microsatellite marker (pchgms1) 12.5 cM from the *br* locus. This microsatellite marker was sequenced and primers produced for PCR amplification.

PCR Amplification and Analysis

The PCR reaction had a total volume of 50 µl. The mixture contained 1x PCR buffer (10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$), 0.2 mM of each dNTP, 0.12 µM of each primer, 0.5 U of DNA polymerase (Fisher-Biotech*Taq*), and 20-50 ng of peach genomic DNA. The PCR reaction was performed in Delta Cycler II System (Ericomp Inc., San Diego). The PCR program had an initial denaturation step at 95 ^{o}C for 5 minutes. This was followed by an additional 30 cycles with denaturation at 94 ^{o}C for 1 minute, annealing at 55 ^{o}C for 1.5 minutes, extension at 72^{o}C for 2 minutes. An additional 5 cycles of denaturation, annealing, and extension at 72^{o}C for 4 minutes was performed to obtain complete PCR product extension. A 10 µl sample of the PCR product was analyzed on a 1.25% agarose gel using 1x TAE buffer and visualized with ethidium bromide. The rest of the PCR sample was stored at 4 ^{o}C for analysis. The PCR products were further analyzed on a 6% polyacrylamide gel containing 7 M urea. The products were separated using 1x TBE buffer for 30-40 minutes at 70 Volts. After electrophoresis, the gel was processed and silver stained (Stratagene's CastAway TM silver staining kit) using the manufacturer's protocols. The gel was soaked in 10% Glacial-Acetic Acid Fix/Stop solution until tracking dyes were barely visible, rinsed in water for 30 seconds to 1 minute, 30 minute of 1% Enhancing Solution (sodium carbonate, sodium thiosulfate,

and formaldehyde), and dried on a gel dryer.

RESULTS AND DISCUSSION

Comparison of SFP, DW, CT, and ST Genotypes

Evaluation of SFP, DW, CT and ST trees demonstrated that one fragment at approximately 110 bp (upper fragment) was consistently associated with SFP trees (Fig 2). A second, lower fragment at approximately 90 bp was associated with all other growth habits tested including CT, DW, and ST.

Comparison of SFP and NSP Trees

Two populations of hybrid progeny (64 trees, 11 trees) resulted from crosses of red-leaf SFP x green-leaf NSP. All progeny, whether green- or red-leaf were columnar, suggesting that the columnar growth habit from SFP and NSP resulted from the expression of the same gene (Scorza et al., 2002). In contrast to the results of this classic test of allelism, NSP samples did not produce the upper fragment as found in SFP, but only the lower fragment as was produced by all other growth habits tested. Hybrid trees produced from crosses of NSP x SFP and hybrids between SFP and other growth habits displayed both fragments (Fig. 2). Since the origins of SFP and NSP are obscure, the basis of the differences between these two columnar growth habit sources is not known. At least two explanations may be put forward for the presence of the upper fragment in the SFP trees and its absence in the NSP trees. If both the NSP and SFP trees originated from the same germplasm source, the difference in fragment presence could be explained by genetic cross-overs in the 12 CM region between the *br* allele and pchgms1. This would produce NSP without the upper fragment and a non-columnar tree with the upper fragment. Alternatively, if the same mutation to columnar growth habit was produced in two distinct sources of germplasm (NSP and SFP), pchgms1, and hence the upper fragment, could have been present in one genetic background but not the other.

CONCLUSIONS

Peach trees expressing SFP could be selected from all other growth types tested utilizing the pchgms1 microsatellite PCR-based marker. NSP trees, also expressing the columnar growth habit, did not display the fragment particular for SFP trees nor could NSP be distinguished from other growth habits using pchgms1. This discrepancy in distinguishing columnar growth habit produced by the same allele in two lines clearly expressing the columnar trait could be due to the presence of crossovers between the marker and the *br* allele, or the mutation that produced the *br* allele could have occurred in distinctly different germplasm sources with the microsatellite pchgms1 being present in only one genetic background. The development of markers more closely linked to the *br* allele will be necessary in order to more effectively use marker-assisted selection for columnar growth habit.

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Literature Cited

Scorza, R., Bassi, D. and Liverani, A. 2002. Genetic interactions of pillar (columnar), compact, and dwarf peach tree genotypes. J. Amer. Soc. Hort. Sci. 127:254-261.

Sosinski, B., Gannavarapu, M., Hager, L.D., Beck, L.E., King, G.J., Ryder, C.D., Rajapaskse, S., Baird, W.V., Ballard, R.E. and Abbott, A.G. 2000. Characterization of microsatellite markers in peach [Prunus persica (L.) Batsch]. Theor. Appl. Genet. 101:421-428.

Yamazaki, K., Okabe, M. and Takahashi, E. 1987. Inheritance of some characteristics and breeding of new hybrids in flowering peaches. Kangawa Enshi Kempo 34:46-53 (Japanese).

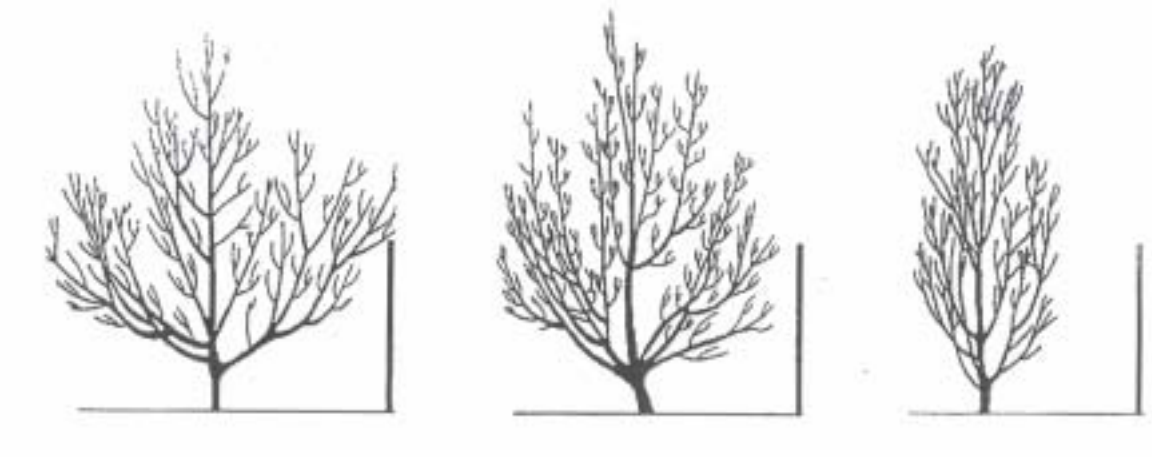

Figure 1. Diagram of standard (ST) (*BrBr*) (left), upright (UP) (*Brbr*) (middle), and columnar (*brbr*) (right) peach trees.

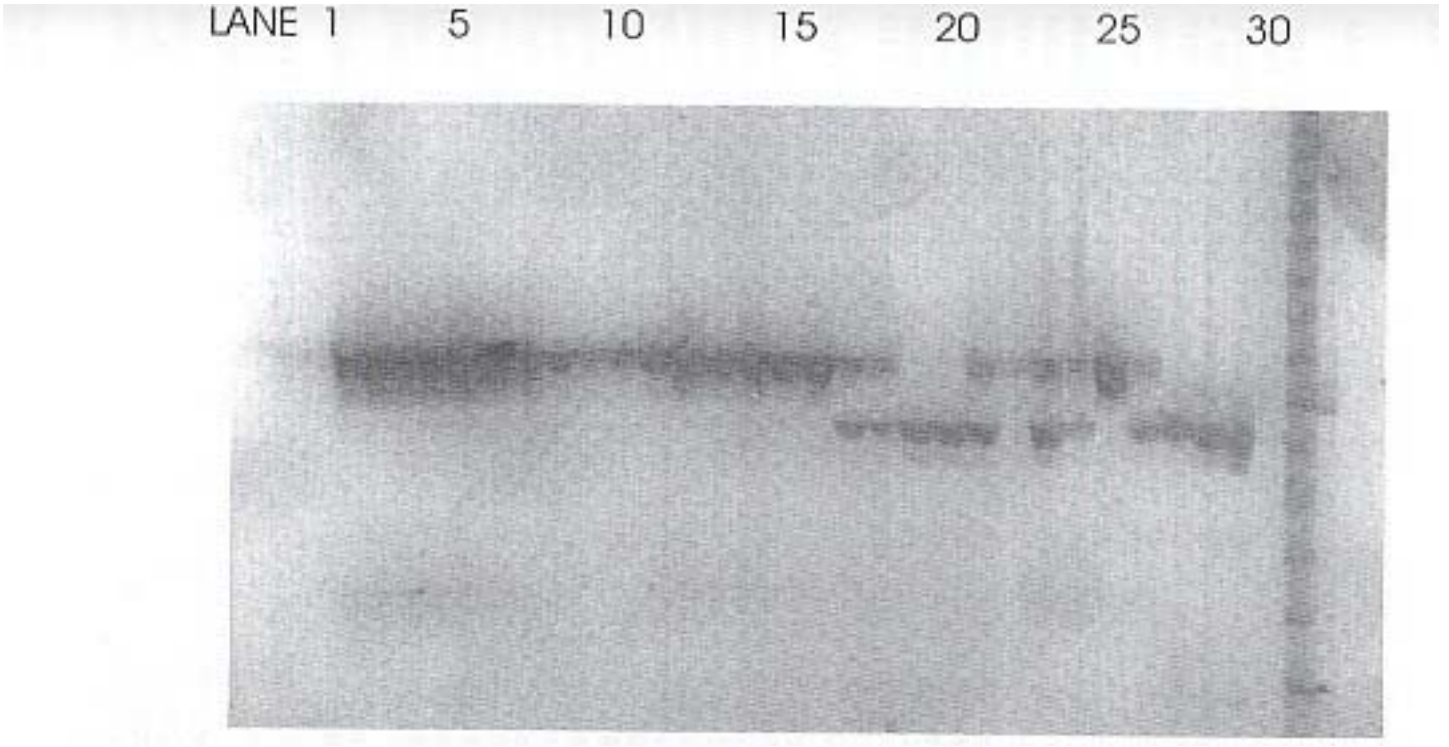

Fig. 2. PCR products from amplification of pchgms 1 separated on a 6% polyacrylamide gel containing 7 M urea. The products were separated using 1x TBE buffer for 30-40 minutes at 70 Volts. After electrophoresis, the gel was processed and silver stained. The upper fragment at approximately 110 bp was consistently associated with SFP trees (lanes 1-16, 22) (molecular markers, lane 31). The lower fragment at approximately 90bp was associated with all other growth habits tested including CT, DW, and ST (lanes 19, 20, 27, 28, 29). Both bands were produced from hybrids of SFP with other growth habits including the columnar phenotype NSP (lanes 17, 18, 21, 23, 24, 26).